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14. ABSTRACT

Military personnel are at a higher risk of developing pancreatic cancer, which is the most lethal common malignancy. Accordingly, our proposed work will first address the focus areas concerning the susceptibility of military personnel to pancreatic cancer following occupational exposure to chemical carcinogens and the recreational use of tobacco products. We hypothesize that carcinogens present in diesel fuel exhaust and cigarette smoke contribute to the higher rates of pancreatic cancer in military personnel. This heightened risk is due in part to the elevated exposure to carcinogens present in diesel engine exhaust and cigarette smoke, such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals. Understanding the susceptibility of military personnel to pancreatic carcinogenesis in tractable model systems will directly lead to the discovery of new biomarkers of pancreatic cancer such that early detection strategies can be developed. Therefore, we will also focus on discovery of biomarkers for carcinogen-induced pancreatic cancer. Along these lines, we aim to first examine the contribution of carcinogens to pancreatic tumorigenesis at levels that model the chronic exposure seen by military personnel. To accomplish this task, we developed a novel, three-dimensional culture systems and defined its engraftment and growth kinetics in a transplantation model. Furthermore, we identified biomarkers of early stage pancreatic cancer and are currently testing whether they will facilitate early detection in at risk populations, such as military personnel and their families.

15. SUBJECT TERMS
Pancreatic ductal adenocarcinoma, early detection, carcinogenesis, military, diesel engine exhaust, cigarette smoke, organoids

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INTRODUCTION: Military personnel are at a higher risk of developing pancreatic cancer, which is the most lethal common malignancy. Accordingly, our proposed work will first address the focus areas concerning the susceptibility of military personnel to pancreatic cancer following occupational exposure to chemical carcinogens and the recreational use of tobacco products. We hypothesize that carcinogens present in diesel fuel exhaust and cigarette smoke contribute to the higher rates of pancreatic cancer in military personnel. This heightened risk is due in part to the elevated exposure to carcinogens present in diesel engine exhaust and cigarette smoke, such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals. Understanding the susceptibility of military personnel to pancreatic carcinogenesis in tractable model systems will directly lead to the discovery of new biomarkers of pancreatic cancer such that early detection strategies can be developed. Therefore, we will also focus on discovery of biomarkers for carcinogen-induced pancreatic cancer. Along these lines, we aim to first examine the contribution of carcinogens to pancreatic tumorigenesis at levels that model the chronic exposure seen by military personnel. Furthermore, we will use novel, three-dimensional culture systems to identify biomarkers of early stage pancreatic cancer that facilitate early detection in at risk populations, such as military personnel and their families.

KEYWORDS: Pancreatic ductal adenocarcinoma, early detection, carcinogenesis, military, diesel engine exhaust, cigarette smoke, organoids.

ACCOMPLISHMENTS:

What were the major goals of the project?

Task	Months	Status
Major Task 1 - Investigate the role of carcinogens on pancreas carcinogenesis on mouse organoids		
Subtask 1: Generate mouse N, P, and T organoids	1-4	Completed
Subtask 2: Orthotopic transplantation of mouse organoids into syngeneic mice and treatment with carcinogens (benzo(a)pyrene and/or Cadmium)	2-6	Baseline completed, established growth kinetics of transplanted organoids without exogenous carcinogenesis, carcinogenesis of organoids in progress
Subtask 3: Monitor tumor growth and prepare formalin-fixed and snap frozen tissues	2-12	
Subtask 4: Histological Analysis from mouse organoids samples	2-12	
Subtask 5: Perform DNA and RNA sequencing and analyze the data	12-16	Not initiated yet
Subtask 6: Validate candidate genes in vitro	16-24	
Major Task 2 - Investigate the role of carcinogens on pancreas carcinogenesis on human organoids		
Subtask 1: Optimize human N, P and T organoid culture system	1-6	Completed
Subtask 2: Mutate *Kras* in human normal organoids by gene editing	1-8	In progress
Subtask 3: Orthotopic transplantation of human organoids into immunocompromised mice and treatment with carcinogens (benzo(a)pyrene and/or Cadmium)	2-8	Baseline completed, established growth kinetics of transplanted organoids without exogenous carcinogenesis, carcinogenesis of organoids in progress
Subtask 4: Monitor tumor growth and prepare formalin-fixed and snap frozen tissues	2-16	
Subtask 5: Histological analysis of human organoid samples	3-18	
Major Task 3 – Identify specific biomarkers of pancreatic cancer using the mouse organoid system		
Subtask 1: Generate mouse organoids that produce CA19-9	1-2	Completed
Subtask 2: Orthotopic transplantation of mouse organoids into syngeneic mice and treatment with carcinogens (benzo(a)pyrene and/or Cadmium) and	2-6	Baseline completed, established growth kinetics of transplanted organoids without exogenous carcinogenesis

Cerulein.		
Subtask 3: Monitor tumor growth and collect plasma and ascites from mice implanted with mouse organoids	3-12	Baseline completed, established growth kinetics of transplanted organoids without exogenous carcinogenesis
Subtask 4: Analyze CA19-9 carriers by immunoprecipitation and mass spectrometry	6-18	CA19-9 IP/MS optimized and completed on mouse organoid lysates in the absence of exogenous perturbation
Subtask 5: Validate candidate CA19-9 carrier proteins by immunohistochemistry on mouse tumor samples and protein levels in the blood during tumor progression	18-24	
Major Task 4 – Identify specific biomarkers of pancreatic cancer using the human organoid system		
Subtask 1: Determine the ability of human organoids to produce CA19-9	1-8	Completed
Subtask 2: Orthotopic transplantation of human organoids into immunocompromised mice and treatment with carcinogens (benzo(a)pyrene and/or Cadmium)	2-8	Baseline completed, established growth kinetics of transplanted organoids without exogenous carcinogenesis
Subtask 3: Monitor tumor growth and collect plasma and ascites from mice implanted with human organoids	2-16	
Subtask 4: Analyze CA19-9 carriers by immunoprecipitation and mass spectrometry	6-18	CA19-9 IP/MS optimized and completed on human organoid lysates and human patient sera in the absence of exogenous perturbation, proof of concept validation by reciprocal co-IP on novel carriers
Subtask 5: Validate candidate CA19-9 carrier proteins by immunohistochemistry on human tumor samples and protein levels in the blood during tumor progression	18-24	

What was accomplished under these goals?

We established organoid models from normal and neoplastic murine and human pancreas tissues. Pancreatic organoids can be rapidly generated from resected tumors and biopsies, survive cryopreservation and exhibit ductal- and disease stage-specific characteristics. Orthotopically transplanted neoplastic organoids recapitulate the full spectrum of tumor development by forming early-grade neoplasms that progress to locally invasive and metastatic carcinomas. Due to their ability to be genetically manipulated, organoids are a platform to probe genetic cooperation. Comprehensive transcriptional and proteomic analyses of murine pancreatic organoids revealed new genes and pathways altered during disease progression. The confirmation of many of these protein changes in human tissues demonstrates that organoids are a facile model system to discover characteristics of this deadly malignancy. This work culminated in a publication in Cell (see below).

Major tasks 1 and 2: Investigate the role of carcinogens on pancreas carcinogenesis on mouse and human organoids.

To identify the novel drivers that are triggered by exposure to components of cigarette smoke and diesel exhaust, we proposed to employ an orthotopically grafted organoid (OGO) model using mouse mN, mP, and mT organoids expressing luciferase and mCherry. We have generated three independent organoid lines expressing luciferase, mCherry and puromycin as a selection marker using lentivirus and tested the tumor growth kinetics of these transplanted organoids. Unlike the three unmodified mouse T organoids that developed tumors within 3-4 months post-transplant, only two out of 11 organoids (3-4 mice for 3 independent mT organoids) expressing luciferase, mCherry and puromycin developed tumors in 3 months. These differences in engraftment and growth kinetics could be attributed to the immune response towards foreign proteins (mCherry, Luciferase, Puromycin) in syngeneic mice. Therefore, we are currently employing unmodified mouse organoids for transplantation experiments for carcinogenesis.

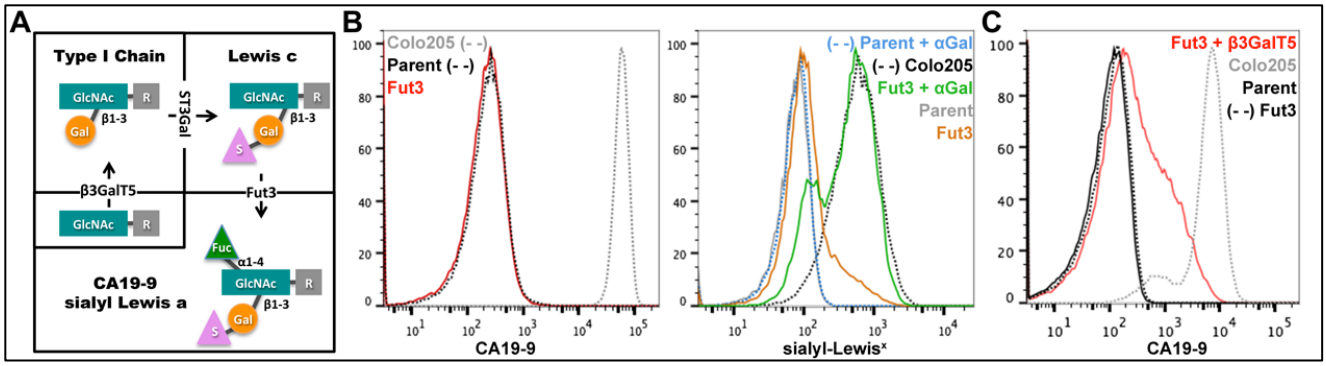

Figure 1. Fut3 and β3GalT5 are required for CA19-9 production in mouse pancreatic cancer cells. A. Enzymatic generation of CA19-9. **B.** Flow cytometric demonstration that Fut3 is functional, but not sufficient to make CA19-9. Colo205: Colorectal cell line, positive control. Parent: Mouse PDAC cells. **C.** Expression of Fut3 and β3GalT5 is sufficient for CA19-9 production.

In the second major task, we proposed to employ human OGO model for carcinogenesis. We established human normal organoids as well as tumor organoids and tested the growth kinetics of these human organoids (hN and hT organoids). Only two out of the 23 transplanted human N organoids were able to integrate into mouse pancreas in immunocompromised mice (ie, nude and NSG mice), which were detected by IHC using a human mitochondrial antibody. Therefore, we attempted to introduce KrasG12D knock-in allele into human N organoids by insertion of synthetic oligonucleotide duplexes (generous gift from Hans Clever's lab) using CRISPR/Cas9. Because there is no selection marker for CRISPR/Cas9 and hN organoids can't survive without EGF supplement in culture media, we have been using EGF deficient media to select KrasG12D knock-in clones. This experiment is currently in progress. As we generate human N organoids expressing KrasG12D, we will transplant them for carcinogenesis transplant experiments.

Major Task 3 and 4; Identify specific biomarkers of pancreatic cancer using the mouse and human organoid system.

To identify changes in CA19-9 carriers in organoid and mouse models of disease progression, we developed a strategy to re-introduce the requisite enzymes responsible for CA19-9 production. Initial experiments were carried out using monolayer cultures of mouse PDAC cells to determine which enzymes are required for CA19-9 production (**Fig. 1A**). Ectopic expression of Fut3 in several mouse PDAC cell lines was insufficient to induce CA19-9 expression, but was functional given its ability to induce production of related Lewis X antigens after unmasking by α-galactosidase (**Fig. 1B**). To redirect Lewis antigen expression to CA19-9 (sialyl Lewis A), we introduced an additional enzyme, β1,3-Galactosyltrasferase 5 (β3GalT5). This enzyme is required for the production of CA19-9 precursor substrates. The combination of these two enzymes enabled production of CA19-9 in five different mouse PDAC cell lines (**Fig. 1C,** data not shown). The production of

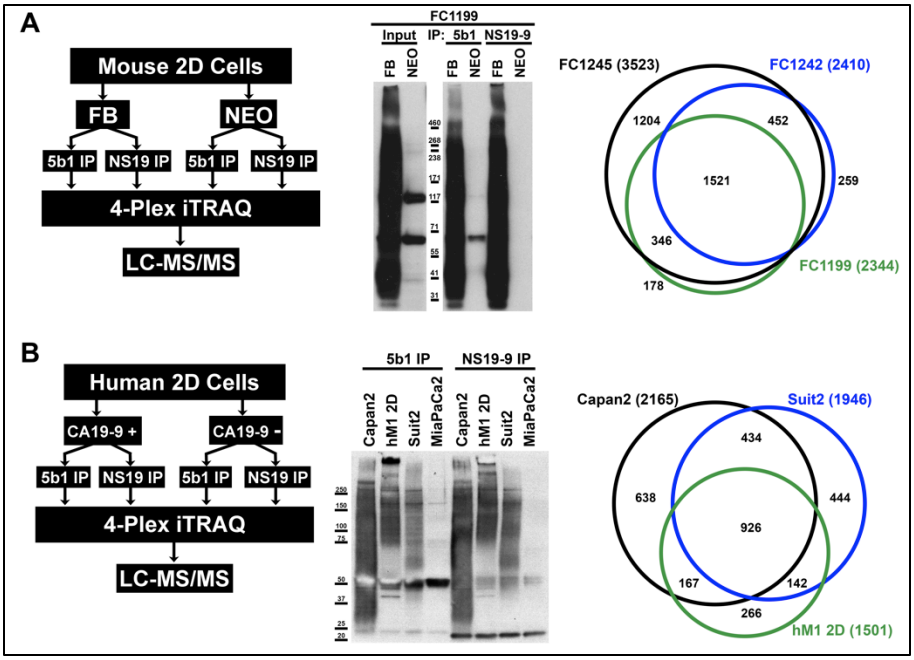

Figure 2. Identification of CA19-9 carriers in monolayer cultures of human and mouse PDAC cells. A. Identification strategy for mouse CA19-9 carriers in cells expressing Fut3 and β3GalT5 (FB). Representative CA19-9 western blot of immunoprecipitation (IP) shown. **B.** Identification strategy for human CA19-9 carriers and representative IP. Overlap for both mouse and human carriers generated by calling CA19-9 carriers as 1.5-fold up-regulated in CA19-9 positive sample relative to the CA19-9 negative control.

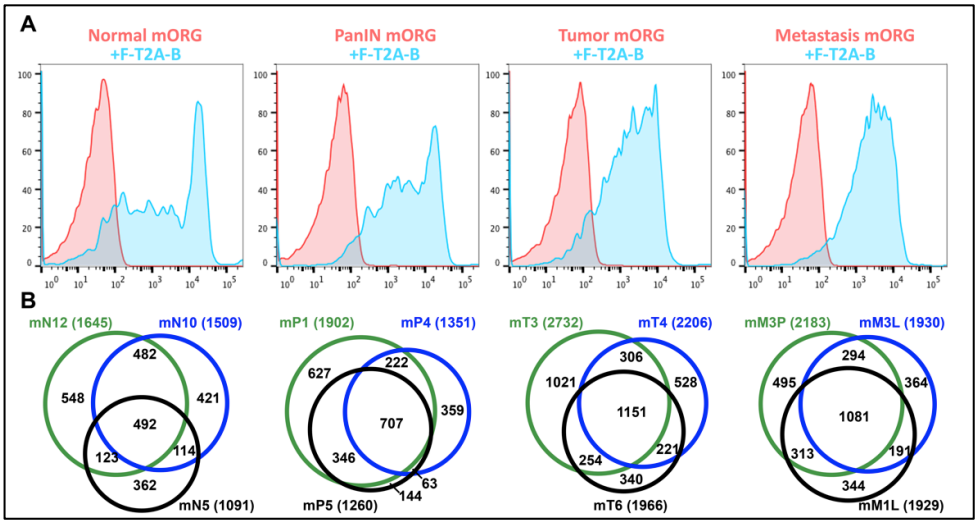

Figure 3. Identification of CA19-9 carriers during each stage of PDAC progression. A. Introduction of FB into mouse organoids (mORG) enables CA19-9 production at each stage of disease. Empty vector- transduced (red) and F-T2A-B- transduced organoids (blue) were evaluated for CA19-9 levels by flow cytometry. **B.** Overlap between CA19-9 carriers identified in three lines of Normal (mN), PanIN (mP), Tumor (mT), and Metastasis (mM) mouse organoids.

CA19-9 was evaluated *in vivo* by orthotopically transplanting these cell lines into syngeneic mice. CA19-9 was detected in the tumors, metastases, and blood of mice that received cells expressing **F**ut3 and **β**3GalT5 (FB), while none was detected in control mice receiving pBabe-neo infected cells (data not shown).

The CA19-9 carriers between mouse and human cells were compared to discern whether CA19-9 is added to known carriers in the genetically engineered mouse system. CA19-9 labeled proteins were immunoprecipitated (IP) and identified by mass spectrometric comparison of the CA19-9 expressing cells (FB) to empty-vector transduced controls (Neo) (**Fig. 2A**). Differences in relative protein abundance between CA19-9 positive and negative cells were determined using isobaric Tags for Relative and Absolute Quantification (iTRAQ) and mass spectrometry (MS) (1). Two CA19-9 antibody clones were selected based on their use in the literature (5b1, NS19-9) (2, 3). CA19-9 carriers were defined as up-regulated at least 1.5-fold in the CA19-9 positive relative to negative samples using either CA19-9 antibody. These analyses identified known CA19-9 carriers in the FB mouse cells, including Muc1, CD44 and Muc5ac. The human PDAC cell line, MiaPaCa-2, is CA19-9 negative and used as a negative control (**Fig. 2B**). We compared mouse and human CA19-9 carriers and found 65% of the mouse carriers were present in human cells. These data suggest that FB expression in mouse cells recapitulates the human CA19-9 carrier profile.

During the current reporting period, we engineered mouse normal (mN), PanIN (mP), tumor (mT), and metastasis (mM) organoids to express CA19-9 (**Fig. 3A**) and CA19-9 carriers were identified as described above (**Fig. 2A and 3B**). CA19-9 carriers identified in at least 2 out of 3 mP, mT, and mM organoid cultures were compared to those found in any mN organoid culture. Approximately 78% of the tumor CA19-9 carriers are also present in at least one normal organoid culture, including Muc1 and CD44 (**Fig. 4**) (4-7). Upon subtraction of mN CA19-9 carriers, 844 protein carriers remained that were found uniquely in mP, mT, and/or mM organoids. 67% (565) of the CA19-9 carriers specific to transformation of the pancreas have been published to enter the circulation and represent potential biomarkers of PDAC (8).

We also identified PDAC-specific CA19-9 carriers using human normal (hN) and tumor (hT) organoids

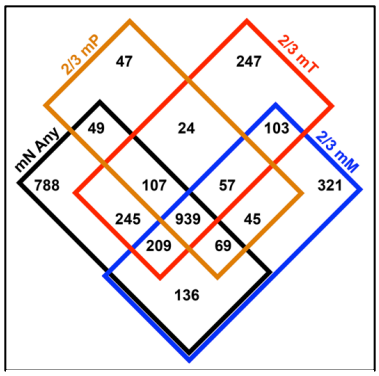

Figure 4. Overlap between mouse organoid CA19-9 carriers.

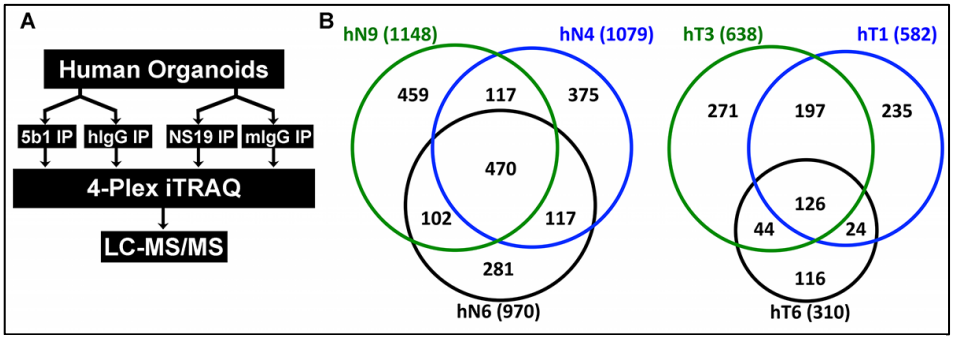

Figure 5. Identification of CA19-9 carriers in human organoids. A. Identification strategy. **B.** Overlap of CA19-9 carriers identified in hN and hT organoids. CA19-9 carriers are defined as being up-regulated >1.5-fold in the CA19-9 relative to the IgG IP using either CA19-9 antibody.

(9). CA19-9 negative individuals represent approximately 10% of the Caucasian population (10), but our human organoid cultures have not yet included a CA19-9 negative specimen. Therefore, instead of using CA19-9 negative cells, isotype-matched control antibodies (hIgG, mIgG) were used as a reference for CA19-9 carrier identification (**Fig. 5A-B**). This approach identified known CA19-9 carriers, such as MUC1, MUC5AC, CD44, and LGALS3BP (4-7). Approximately 10% of the CA19-9 carriers identified in hT organoids were unique to this malignant state (**Fig. 6**). MUC13 was found in three hT organoids, but was absent in all hN cultures. MUC13 IP from human 2D PDAC cell lysates and conditioned media (**Fig. 7,** data not shown) validated Muc13 as a CA19-9 carrier. In addition, we and others demonstrated MUC13 elevation in PDAC (data not shown) (11). Preliminary experiments identifying CA19-9 carriers in serum pools from patients with PDAC or benign disease corroborate these findings and suggest that this approach accurately predicts biomarkers that will be confounded by non-malignant proliferative conditions (**Fig. 8**, data not shown).

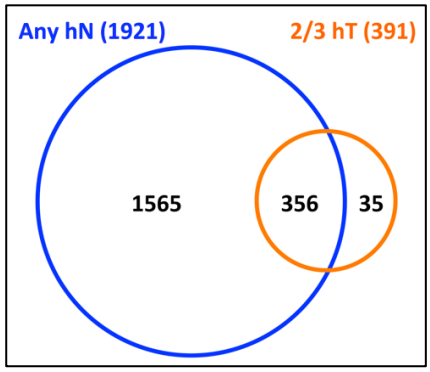

Figure 6. Overlap between hN and hT CA19-9 carriers. Comparison of CA19-9 carriers identified in any of the hN and 2 out of 3 hT cultures.

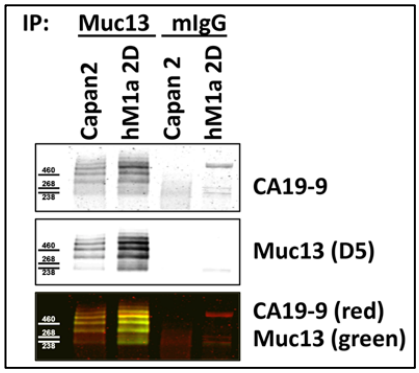

Figure 7. Validation of Muc13 as a novel CA19-9 carrier. Immunoblot of Muc13 IP (H300) and isotype (mIgG) IP with CA19-9 and Muc13 (D5).

	PathHistology	Notes	Gender
PDAC Pool	Adenocarcinoma, Ductal	TBILI 0.8, CA 199 109, CEA 3	F
	Adenocarcinoma, Ductal	TBILI 2.8, CA 199 1687, CEA 4.4	F
	Adenocarcinoma, Ductal	TBILI 6.9, CA 199 313, CEA 3.5	F
	Adenocarcinoma, Ductal	TBILI 1.4, CA 199 566, CEA 3.9	M
	Adenocarcinoma, Ductal	TBILI 0.5, CA 199 1206, CEA 5.3	M
Benign Pool	chronic pancreatitis	TBILI 6.8, CA 199 37	M
	chronic pancreatitis	TBILI 2.4, CA 199 74, CEA 1.4	M
	SCA	TBILI 0.4, CA 199 38, CEA 2.3	F
	benign retention cyst	TBILI 6.5, CA 199 169, CEA 6.4	F
	SCA	TBILI 1.1, CA 199 75, CEA 2.2	M

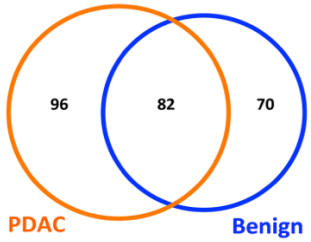

Figure 8. Identification of CA19-9 carriers in patient sera reveals overlap between PDAC and benign disease. Left. PDAC and benign pancreatic disease pools of patient sera. **Right.** Overlap between CA19-9 carriers in PDAC and Benign disease.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

Major Tasks 1 and 2: We will continue to transplant mouse organoids (P and T) and human T organoids to be treated with carcinogens. The unmodified mouse P and T organoids without any genetic manipulation *in vitro* are being used for these experiments. Additionally, as we select successfully hN organoids expressing KrasG12D, we will transplant them immediately. The latest end points of experiments to collect samples will be 8 months for transplanted P organoids and 2 months for transplanted T organoids post transplant unless these carcinogen-treated mice develop tumors sooner than the control group. As described in the pitfall and alternative section, we propose to treat organoids with these carcinogens in vitro first prior to transplantation. Post-transplantation, we will also treat mice with carcinogens as described in our original proposal. Also we propose to employ mP and mT organoids not mN organoids since normal organoids were unable to be integrated into the pancreas in vivo. Since the growth kinetics of transplanted organoids were assessed, we don't anticipate any difficulties to complete these tasks by the end of the next reporting period.

Major Tasks 3 and 4: We established the organoids expressing CA19-9 and most importantly, optimized CA19-9 IP/MS on mouse and human organoids lysates during the current reporting period. Therefore, we will focus on

OGO experiments during the next reporting period to complete the analysis of CA19-9 IP/MS using plasma collected from carcinogen-treated transplanted mice and identify potential carcinogen-induced biomarkers.

In addition, as described in the pitfall and alternative section, we will utilize Multiple Reaction Monitoring (MRM) to measure CA19-9 carrier abundance in patient sera because suitable antibodies are unavailable for many of the biomarker candidates. MRM is a multiplexed MS technique to quantify protein concentrations using stable isotope labeled standard peptides, and has been successfully implemented to measure plasma and serum biomarkers for pancreatic and other cancers (12). We will develop MRM assays for the human equivalents of the CA19-9 carriers that discriminate between normal and malignant mouse organoids and that can be detected in the circulation. We will prioritize carriers that are found early in disease progression (mP) and are also present in later stages of tumor progression (mT). CA19-9 carriers that are found in hT, but never detected in hN organoids, will be also investigated. In addition, we will include a cohort of canonical CA19-9 carriers that are found in both normal and malignant proliferative states, such as CD44 and LGALS3BP. We will determine the concentration of the selected CA19-9 carriers in blinded, individual patient samples that were diagnosed with benign pancreatic disease or PDAC. These analyses will first involve the IP of CA19-9 carriers and then their identification by MS (**Fig. 9A**). A pilot of the MS-based quantitation yielded promising candidates and validated the methodology in patient sera pools (**Fig. 9B**).

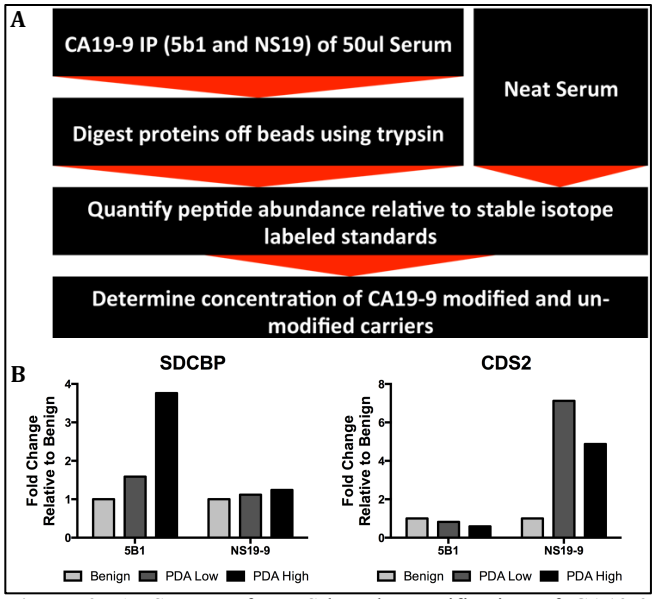

Figure 9. A. Strategy for MS-based quantification of CA19-9 modified carriers and their total abundance. **B.** Example quantitation of two CA19-9 carriers in sera pools from patients with Benign disease or PDAC with low or high CA19-9 levels.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Task	Proposed changes
Major Task 1 - Investigate the role of carcinogens on pancreas carcinogenesis on mouse organoids	
Subtask 1: Generate mouse N, P, and T organoids	No changes
Subtask 2: Orthotopic transplantation of mouse organoids into syngeneic mice and treatment with carcinogens (benzo(a)pyrene	Whole animal carcinogenesis is likely to yield non-pancreatic tumors, resulting in a large increase in the number of mice needed in order to generate the requisite number of animals with

and/or Cadmium)	pancreatic lesions. Therefore, as suggested in our pitfalls and alternatives section, we will treat the organoids with these carcinogens prior to implantation.
Subtask 3: Monitor tumor growth and prepare formalin-fixed and snap frozen tissues	No changes
Subtask 4: Histological Analysis from mouse organoids samples	No changes
Subtask 5: Perform DNA and RNA sequencing and analyze the data	No changes
Subtask 6: Validate candidate genes in vitro	No changes
Major Task 2 - Investigate the role of carcinogens on pancreas carcinogenesis on human organoids	
Subtask 1: Optimize human N, P and T organoid culture system	No changes
Subtask 2: Mutate *Kras* in human normal organoids by gene editing	No changes
Subtask 3: Orthotopic transplantation of human organoids into immunocompromised mice and treatment with carcinogens (benzo(a)pyrene and/or Cadmium)	Whole animal carcinogenesis is likely to yield non-pancreatic tumors, resulting in a large increase in the number of mice needed in order to generate the requisite number of animals with pancreatic lesions. Therefore, as suggested in our pitfalls and alternatives section, we will treat the organoids with these carcinogens prior to implantation.
Subtask 4: Monitor tumor growth and prepare formalin-fixed and snap frozen tissues	No changes
Subtask 5: Histological analysis of human organoid samples	No changes
Major Task 3 – Identify specific biomarkers of pancreatic cancer using the mouse organoid system	
Subtask 1: Generate mouse organoids that produce CA19-9	No changes
Subtask 2: Orthotopic transplantation of mouse organoids into syngeneic mice and treatment with carcinogens (benzo(a)pyrene and/or Cadmium) and Cerulein.	Whole animal carcinogenesis is likely to yield non-pancreatic tumors, resulting in a large increase in the number of mice needed in order to generate the requisite number of animals with pancreatic lesions. Therefore, as suggested in our pitfalls and alternatives section, we will treat the organoids with these carcinogens prior to implantation.
Subtask 3: Monitor tumor growth and collect plasma and ascites from mice implanted with mouse organoids	No changes
Subtask 4: Analyze CA19-9 carriers by immunoprecipitation and mass spectrometry	No changes
Subtask 5: Validate candidate CA19-9 carrier proteins by immunohistochemistry on mouse tumor samples and protein levels in the blood during tumor progression	Instead of performing IHC, we will perform MRM assays.
Major Task 4 – Identify specific biomarkers of pancreatic cancer using the human organoid system	
Subtask 1: Determine the ability of human organoids to produce CA19-9	No changes
Subtask 2: Orthotopic transplantation of human organoids into immunocompromised mice and treatment with carcinogens	Whole animal carcinogenesis is likely to yield non-pancreatic tumors, resulting in a large increase in the number of mice needed in order to generate the requisite number of animals with

(benzo(a)pyrene and/or Cadmium)	pancreatic lesions. Therefore, as suggested in our pitfalls and alternatives section, we will treat the organoids with these carcinogens prior to implantation.
Subtask 3: Monitor tumor growth and collect plasma and ascites from mice implanted with human organoids	No changes
Subtask 4: Analyze CA19-9 carriers by immunoprecipitation and mass spectrometry	No changes
Subtask 5: Validate candidate CA19-9 carrier proteins by immunohistochemistry on human tumor samples and protein levels in the blood during tumor progression	No changes

Actual or anticipated problems or delays and actions or plans to resolve them

As we mentioned previously, we proposed to employ an orthotopically grafted organoid (OGO) model using mouse mN, mP, and mT organoids expressing luciferase and mCherry. We have generated three independent organoid lines expressing luciferase, mCherry and puromycin as a selection marker using lentivirus and tested the tumor growth kinetics of these transplanted organoids. Unlike the three unmodified mouse T organoids that developed tumors within 3-4 months post-transplant, only two out of 11 organoids (3-4 mice for 3 independent mT organoids) expressing luciferase, mCherry and puromycin developed tumors in 3 months. These differences in engraftment and growth kinetics could be attributed to the immune response towards foreign proteins (mCherry, Luciferase, Puromycin) in syngeneic mice. Therefore, we are currently employing unmodified mouse organoids for transplantation experiments for carcinogenesis.

These results have slighly delayed systemic carcinogenesis of transplant recipients. Therefore, as reported in previous sections, we plan to expedite our progress by performing carcinogenesis in vitro prior to transplantation as proposed as an alternative in our proposal.

In the second major task, we proposed to employ human OGO model for carcinogenesis. We attempted to introduce KrasG12D knock-in allele into human N organoids by insertion of synthetic oligonucleotide duplexes (generous gift from Hans Clever's lab) using CRISPR/Cas9. Because there is no selection marker for CRISPR/Cas9 and hN organoids can't survive without EGF supplement in culture media, we have been using EGF deficient media to select KrasG12D knock-in clones. This experiment is currently in progress. As we generate human N organoids expressing KrasG12D, we will transplant them for carcinogenesis transplant experiments.

Changes that had a significant impact on expenditures:

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

No changes.

Significant changes in use or care of human subjects: No changes.

Significant changes in use or care of vertebrate animals: No changes.

Significant changes in use of biohazards and/or select agents: No changes.

PRODUCTS: Nothing to report

Publications, conference papers, and presentations:

Journal publications.

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Giulia Biffi, Georgi N. Yordanov, Bethany Delcuze, Brianna Creighton, Kevin Wright, Youngkyu Park, Folkert H.M. Morsink, I. Quintus Molenaar, Inne H. Borel Rinkes, Edwin Cuppen, Yuan Hao, Ying Jin, Isaac J. Nijman, Christine Iacobuzio-Donahue, Steven D. Leach, Darryl J. Pappin, Molly Hammell, David S. Klimstra, Olca Basturk, Ralph H. Hruban, George Johan Offerhaus, Robert G.J. Vries, Hans Clevers, David A. Tuveson. (2015). Organoid Models of Human and Mouse Ductal Pancreatic Cancer. Cell *160*, 324-338. * Co-first authors

- **DoD support was acknowledged**

Books or other non-periodical, one-time publications: Nothing to report

Other publications, conference papers, and presentations: Nothing to report

Website(s) or other Internet site(s): Nothing to report

Technologies or techniques: Nothing to report

Inventions, patent applications, and/or licenses: Nothing to report

Other Products: Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	David Tuveson
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-8017-2712
Nearest person month worked:	1
Contribution to Project:	Dr. Tuveson serves as the Principal Investigator of this project and oversees all aspects of its undertaking, including experimental design and data analysis.
Funding Support:	NIH, AACR, PMRA, Lustgarten Foundation, STARR, DoD, Regents of Univ. Min., CSHL

Name:	Youngkyu Park
Project Role:	Research Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-1169-6739
Nearest person month worked:	6
Contribution to Project:	Dr. Park contributed to the development of the organoid model systems, cloning of the targeting constructs for CRISPR/Cas9 gene editing, generation and validation of the derived organoids, and transplantation into mice.
Funding Support:	DoD, Northshore LIJ

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period: Nothing to report.

What other organizations were involved as partners: Nothing to report.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Nothing to report.

QUAD CHARTS: Nothing to report.

APPENDICES: Nothing to report.

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